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ORIGINAL PAPER

Plant–plant and plant–soil interactions under drought and the presence of invasive buffelgrass (*Cenchrus ciliaris*)

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Abstract Soil microbiomes could mediate plant responses to interacting environmental changes such as drought and plant invasion. We used a greenhouse experiment to assess changes in soil bacterial/archaeal and fungal communities, and competition between buffelgrass (*Cenchrus ciliaris*; an African perennial C_4 bunchgrass introduced in the Sonoran Desert) and two native Sonoran Desert plants (i.e., *Aristida purpurea* and *Plantago patagonica*) under experimental drought. We found that buffelgrass benefitted from growing in the vicinity of native plants, particularly in drier conditions, and that this effect might be mediated by higher bacterial richness and an increased proportion of putative nitrogen-fixing bacteria in surrounding soil. Overall, plant–soil interactions are key to understand the interactive effects of abiotic and biotic stressors, and thus, critical to the management and restoration of invaded ecosystems.

Keywords Buffelgrass · Soil microbiome · Drought · Greenhouse · Sonoran Desert

Introduction

Global climate models forecast precipitation changes, including more intense droughts that will promote the expansion of drylands (Huang et al. 2016). Specifically, the southwest USA is predicted to become more arid with more intense, sporadic precipitation events delivering less water overall (Seager et al. 2007). Altered precipitation regimes can substantially influence plant root-shoot ratios, litter quantity and quality, and changes in root vertical distribution and exudate composition (Preece and Peñuelas 2016; Zhang et al. 2019a). Water availability also regulates soil microorganisms by direct physiological stress and indirectly by limiting diffusion and thus, nutrient supply (Schimel 2018). As a consequence, drought can modify both plant competitive ability and plant–soil interactions (Suttle et al. 2007; Pugnaire et al. 2019). For instance, a trade-off between growth rate and water-use efficiency can influence interspecific interactions in desert annual plants (Huxman et al. 2008), and positive interactions with mycorrhizal fungi can alleviate drought stress on plants (Porter et al. 2020). These drought-driven plant–plant and plant–soil interactions can subsequently result in cascading effects such as biodiversity loss, reduced soil carbon sequestration, increased soil erosion, and altered plant

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invasiveness (Meisner et al. 2013; Berdugo et al. 2020).

Invasive plant species are a threat to biodiversity and ecosystem services globally (Pyšek et al. 2012) and consequently, billions of dollars are spent to manage them (Diagne et al. 2021). Although it is expected that native plants have a competitive advantage over invasives in low-resource environments (Funk 2013), and plant–soil responses to drought are expected to be generally positive when plant and soil microorganisms are co-adapted, that is, they share a history of coexistence (de Vries et al. 2023), invasive grasses in the arid southwest USA have rapidly become dominant (Olsson et al. 2012b). Bufffelgrass (*Cenchrus ciliaris*; syn. *Pennisetum ciliare*), an African perennial C_4 bunchgrass, was introduced in the Sonoran Desert for cattle forage and erosion control in the 1930's (Marshall et al. 2012). The success of buffelgrass outside its native range is due to its low water requirements (Ward et al. 2006), high germination rates (Tinoco-Ojanguren et al. 2016), rapid nitrogen acquisition (Lyons et al. 2013), and its ability to modify native neighboring vegetation through allelopathy (Espinoza et al. 2020). In addition, the presence of buffelgrass has been associated with an idiosyncratic soil microbiome (Gornish et al. 2020). Buffelgrass fills barren gaps between native grasses, shrubs and succulents, and these continuous grass stands promote fire hazards, creating a new fire regime unsuitable for native species (McDonald and McPherson 2013). The altered fire regimes also create favorable conditions for buffelgrass, which recover and spread postfire, maintaining a grass-fire cycle (Fusco et al. 2019). As a consequence, buffelgrass invasion can reduce the number of native plant species by more than 50% (Olsson et al. 2012a; Tinoco-Ojanguren et al. 2013). Although a buffelgrass environmental niche model suggested changes in the present distribution and the invasion of areas previously unaffected (de Albuquerque et al. 2019), this model lacked biotic information related to plant competition and interactions with the surrounding soil microbiome.

Co-occurring stressors such as climate disturbances and biological invasions are significant threats to biodiversity and ecosystem functioning, and their effects can interact either synergistically or antagonistically (Valliere et al. 2017; Fahey et al. 2020). For example, competitive interactions between native and invasive plants can either be disrupted during intense environmental stress (Davis et al. 2000), or invasive plants may mitigate drought effects on natives via habitat modification (Rodriguez 2006). Arid ecosystems in the southwest USA might be particularly susceptible to the interactive effects of these stressors as water availability is the most critical factor regulating biological activity (Schwinning and Sala 2004), and in recent decades non-native grasses are spreading and transforming the fire regime (Fusco et al. 2019). Thus, understanding how drought and native-invasive competition interactively affect plant growth, biomass allocation, and its concomitant influence on soil microbial communities is critical to the management and restoration of invaded arid ecosystems.

In a greenhouse study, we examined competition between buffelgrass and two native Sonoran Desert plants under two water treatments (well-watered vs. drought) by measuring plant traits, and soil microbial diversity and composition. We selected the plant species *Aristida purpurea* (purple three-awn, perennial C_4 grass), and *Plantago patagonica* (woolly plantain, annual forb). Both natives grow in the same habitat as buffelgrass in southern Arizona during the warm season where they are expected to compete strongly; and thus, these natives are commonly used for restoration after buffelgrass removal efforts (Gornish et al. 2020). Our hypotheses were (1) buffelgrass would grow at a high rate even under the drought treatment (Valliere 2019); (2) native plants' biomass allocation to roots would increase with competition and drought (Poorter et al. 2012b); (3) competition treatments (i.e., two different species planted together) would provide a larger diversity of plant resources and thus, would support more diverse soil microbial communities and a higher abundance of fungal saprotrophs (Eisenhauer et al. 2010), and (4) presence of buffelgrass would modify the abundance of nitrogen-cycling soil microorganisms (Gornish et al. 2020). Overall, we aimed to generate an improved mechanistic understanding of plant trait and soil microbial responses to the interactive effects of abiotic and biotic co-occurring stressors.

Methods

Greenhouse experiment

The experiment was conducted in a greenhouse facility at the University of Arizona Campus Agricultural

Center, Tucson, Arizona (USA). Native seeds of *A. purpurea* and *P. patagonica* were purchased from local vendors (Borderland Restoration Network, Patagonia, and Arizona Revegetation and Monitoring Co, Tucson), and buffelgrass seed was hand-collected in the wild (along a roadside in southern Tucson). Plants were germinated in an 8*16 germination tray with approximately 2.5 cm wide pools of 5 cm depth filled with potting soil (Espoma Organic Potting Mix, The Espoma Company, Millville, New Jersey, USA). The tray was initially well-watered by hand to avoid disturbance of the sowed seeds, then an automatic mister was set for 15 s every 15 min for 5 weeks (following Farrell et al. 2022). Upon adequate germination wherein the plants entered the fast-growing vegetative stage, the plants were thinned into individual specimens. We filled 60 pots (550 mL) with a 2:1 mix of 1 m deep sandy, gravely soil gathered from a local soil pit (Marana, Arizona, USA) so that the soil was largely devoid of plant material and microbial biomass, and perlite. This mix resulted in a standardized soil medium with good drainage emulating desert soil. All pots were filled 2.5 cm below the rim of the pot. Each species was planted alone in a pot (1 plant/pot), and planted together with each of the two other species.

We examined the impact of drought using two watering conditions (well-watered vs. drought). Watering treatments were chosen based on summer monsoon season patterns in the region. That is, the well-watered treatment represented a similar average monsoon year (4 days between watering events) based on historical data from 1950 to 2016, while the drought treatment (8 days between watering events) represented predicted changes in precipitation frequency in the region while not resulting in excessive mortality (Farrell et al. 2022). All watering events were done to soil field capacity. Greenhouse daytime temperature averaged 26 °C with 75% relative humidity and nighttime temperature averaged 20 °C with 40% relative humidity. Each treatment combination had five replicates (6 plant treatments, 2 watering treatments, 5 replicates; n = 60).

Plant and soil collection

Plant and soil samples were collected on January 30th, 2022, 10 weeks after the experiment was initiated. Each individual plant was carefully removed to avoid damaging roots by agitating the soil around the crown and slowly separating the plants if they were intertwined, and the number of flowering culms and aboveground height were measured. Plants were washed of excess soil and debris using brushes, dried on paper towels, and separated at the crown or the location where the roots meet the culms. Shoot and root samples were dried in an oven at 70 °C until constant weight (i.e., approximately 72 h), and weighed for total dry root and shoot biomass measurements. Upon plant collection, bulk soil from within the pots and around the roots was collected (n = 60) and placed into Whirl–Pak bags (Sigma-Aldrich, St. Louis, MO, U.S.A). Soil bags were then kept on ice and immediately stored in a − 80 °C freezer.

Molecular analyses and sequence processing

We used the DNeasy PowerLyzer PowerSoil kit (Qiagen, Hilden, Germany) to extract total genomic DNA following the manufacturer's protocols. To characterize bacterial/archaeal communities, we PCR amplified the V4 hypervariable region of the 16S RNA gene using the primers 515-F (GTGCCAGCMGCCGCGGTAA) and the 806-R (GGACTACHVGGGTWTCTAAT) (Walters et al. 2016). For fungal communities, the first internal transcriber region (ITS1) was PCR amplified using the primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) (Walters et al. 2016). PCR was conducted in 40 µL triplicate reactions per sample, using 3 µl of extracted DNA, 3 µL of each primer, 20 µL of MyFi PCR Mix (Bioline, Taunton, MA, USA), and 11 µL of water. PCR consisted of an initial denaturing step at 95 °C for 1 min, 35 cycles of amplification (95 °C for 15 s, 60 °C for 15 s, and 72 °C for 15 s), and a final elongation step of 72 °C for 3 min. Negative controls were included to detect potential contamination. PCR products were quantified with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Waltham, MA USA), pooled in equimolar concentrations, and sequenced on a 2 × 150 bp Illumina MiSeq platform. Sequencing was conducted at the PANDA Core for Genomics and Microbiome Research, University of Arizona.

Sequence reads were demultiplexed using idemp and processed using the DADA2 pipeline (Callahan et al. 2016). 16S rRNA reads were trimmed, and due to length variation in the ITS region, fungal primers were removed with cutadapt (Martin 2011). Reads that exceeded a maximum error of 2 or more bp were removed. Filtered reads were used to train the error model and to infer amplicon sequence variants (ASVs). Paired-end reads were merged, and chimera sequences were removed. We assigned taxonomy using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007) with the SILVA database (Quast et al. 2013) for 16S ASVs and the UNITE database (Nilsson et al. 2019) for ITS ASVs. Any 16S ASVs without a bacterial or archaeal domain classification, or classified as mitochondria or chloroplasts were removed. ITS ASVs without a fungal domain assignment were removed. The number of bacterial/archaeal sequences per sample ranged from 37,623 to 305,825. Fungal sequences per sample ranged from 4418 to 82,435. Functional groups of Bacteria/Archaea were inferred using FAPROTAX (Louca et al. 2016) and fungal guilds with a "highly probable" or "probable" confidence ranking were inferred using FUNGuild (Nguyen et al. 2016). Sequencing data have been deposited in NCBI Sequencing Read Archive (SRA) under BioProject accession PRJNA.984306.

Statistical analyses

All data analyses and visualizations were performed in R version 4.3.0. We explored the associations among plant traits (i.e., height, number of flowering culms, total dry biomass, total root biomass, and root-shoot ratio) using principal component analysis (PCA). We assessed plant and drought treatment differences in plant traits using linear models. We calculated a competitive response index (C_r) by dividing the mean total dry biomass in the presence of neighboring individuals of a different species to the mean total dry biomass in monoculture. A higher C_r value indicates a stronger ability to resist competitive suppression (Goldberg and Fleetwood 1987).

To study soil microbial diversity, we rarefied sequence counts to 35,000 for Bacteria/Archaea and 4400 for Fungi to control for differences in sequencing depth. We assessed the effect of plant and drought treatments on soil microbial richness (number of different ASVs) and diversity (measured using the Shannon index) using linear models. To evaluate soil microbial compositional changes, we calculated Bray–Curtis dissimilarities. Non-metric multidimensional scaling (NMDS) was used to visualize differences in microbial composition, and permutational multivariate analysis of variance (PERMANOVA) was used to evaluate the effect of the plant and drought treatments (Anderson 2001). Multivariate statistics were performed using the vegan package version 2.6-4. Microbial functional group differences among treatments were tested using generalized linear models with negative binomial error structures due to overdispersion and log link functions including the total number of sequences as offset in the MASS package version 7.3-58.4.

Results

Buffelgrass grew bigger, taller and had more flowering culms in monoculture or in competition than native species, particularly under the drought treatment (Table 1; Fig. 1A; Supp. Fig. 1). Overall, buffelgrass showed a stronger competitive response to *A. purpurea* ($C_r = 2.8$ under drought; $C_r = 2.3$ well-watered) than to *P. patagonica* ($C_r = 2.4$ under drought; $C_r = 1.6$ well-watered). Overall, biomass allocation to roots was not significantly different between the watering treatments, but plant treatments within watering treatments (i.e., interaction) were

Table 1 Effects of plant and watering treatments (and their interaction, P × W) on plant traits

Response	Treatment	F	*P*	R^2
Dry biomass	Plant	15.72	**< 0.001**	0.82
	Watering	12.60	**< 0.001**	
	P × W	1.83	0.117	
Height	Plant	9.13	**< 0.001**	0.84
	Watering	0.09	0.764	
	P × W	3.49	**0.007**	
Flowering culms	Plant	7.11	**< 0.001**	0.75
	Watering	21.47	**< 0.001**	
	P × W	3.71	**0.004**	
Root:shoot	Plant	0.64	0.673	0.43
	Watering	0.00	0.959	
	P × W	4.12	**0.002**	
Root mass	Plant	0.53	0.750	0.53
	Watering	0.08	0.786	
	P × W	3.31	**0.009**	

Bold values indicate significant results ($P < 0.05$)

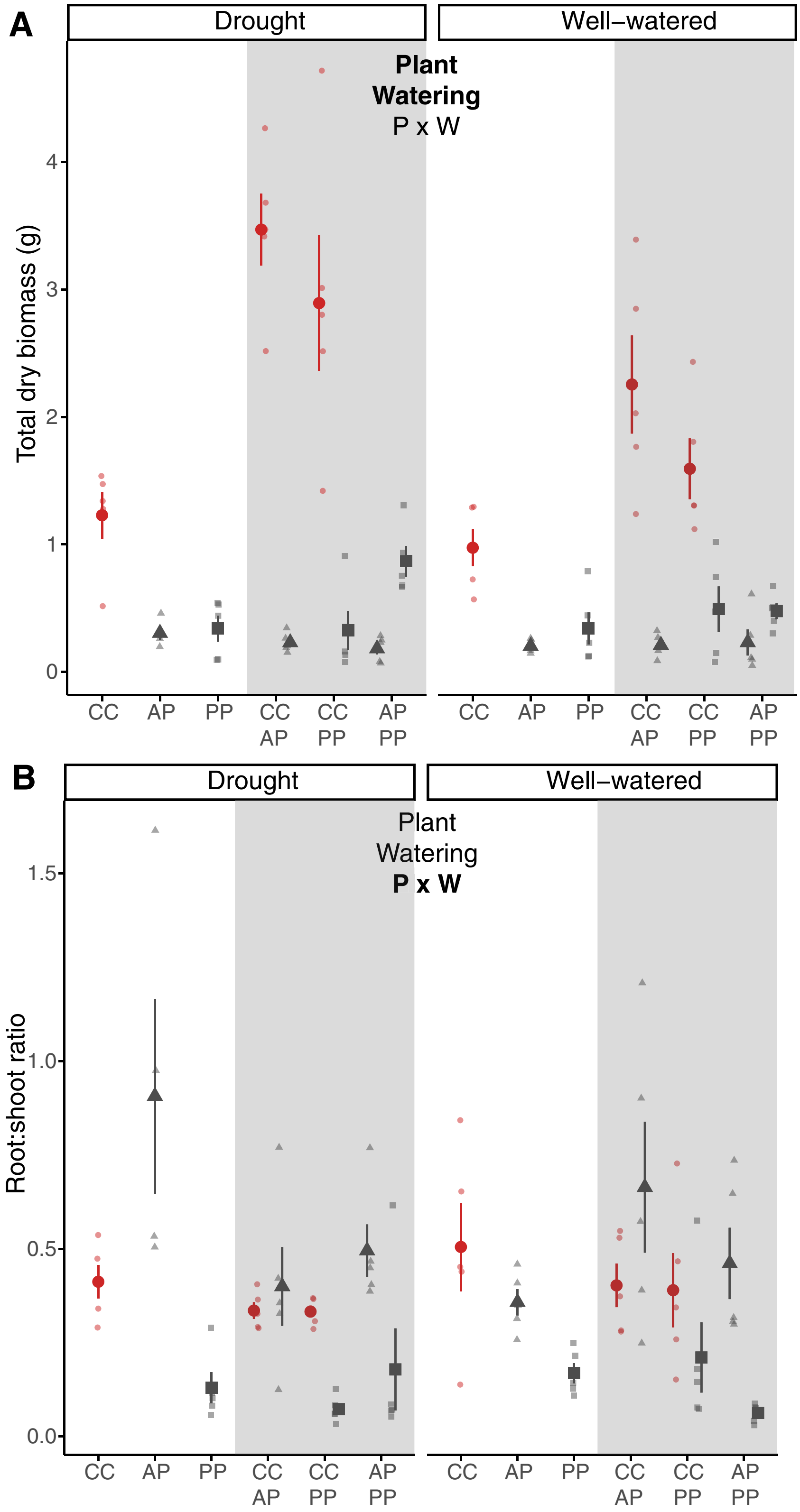


Fig. 1 Total dry plant biomass (**A**) and root:shoot ratio (**B**) across plant and watering treatments. CC refers to *Cenchrus ciliaris* (buffelgrass), AP to *Aristida purpurea* (purple three-awn), and PP to *Plantago patagonica* (woolly plantain). Shaded areas correspond to competition treatments. Bold values indicate significant results ($P < 0.05$) according to statistics in Table 1

significantly different (Table 1; Fig. 1B; Supp. Fig. 1). For instance, when planted alone, *A. purpurea* tended to increase its root biomass allocation under drought conditions, but in competition under drought, root allocation was reduced compared to solo planted (Table 1; Fig. 1B).

The total number of different bacterial/archaeal ASVs after rarefaction was 20,597. Bacterial/archaeal richness per sample ranged from 890 to 2766. The total number of different fungal ASVs was 1001. Fungal richness per sample ranged from 30 to 108. Bacterial/archaeal richness was significantly different among plant treatments (Table 2; Fig. 2A). In particular, plant treatments with any combination of two species had higher bacterial/archaeal richness than monocultures, and pots with buffelgrass (solo or in combination) tended to present higher richness (Fig. 2A). On the contrary, fungal richness did not vary across any treatments (Table 2; Fig. 2B). For both bacterial/archaeal and fungal richness, we did not observe a significant effect of the drought treatment (Table 2; Fig. 2). Similar patterns were observed for Shannon diversity (Supp. Fig. 2).

Soil bacterial/archaeal communities were dominated by Proteobacteria (42.43%), Actinobacteria (25.44%), Bacteroidetes (7.34%), Acidobacteria (3.95%), and Gemmatimonadetes (3.71%). Soil fungal communities were dominated by Ascomycota (80.73%), Olpidiomycota (9.42%), and Basidiomycota (9.28%). Overall soil bacterial/archaeal and fungal compositions were significantly different across plant treatments, watering treatments, and their interaction (Table 2; Supp. Fig. 3). For both Bacteria/Archaea and Fungi, differences across plants explained a larger part of the variation (Table 2). As expected from the results of overall microbial composition, the abundance of most soil microbial functional groups varied significantly across different plant treatments (except for denitrifiers, chitinolytic and cellulolytic bacteria; Table 2). For instance, the abundance of putative fungal plant pathogens under well-watered conditions increased compared to the native monocultures when different species were planted together (Table 2; Fig. 3A). The abundance of putative nitrogen fixers was higher when native plants were planted with buffelgrass only under drought conditions (Table 2; Fig. 3B). The abundance of putative nitrifiers was generally higher in the well-watered treatment (Table 2; Fig. 3C).

Discussion

Effects of drought and competition on buffelgrass' growth and biomass allocation

There exist a myriad of hypotheses to explain plant invasion success and why some species invade certain ecosystems while others fail (Gioria et al. 2023). Although invasive plants tend to be associated with fast return on investment traits (i.e., low leaf mass per unit area, high carbon assimilation rates, short-lived tissues) and thus, are expected to disproportionately succeed in high resource environments, many invaders have specific traits associated with higher resource use efficiency (e.g., C_4 grasses with higher photosynthetic nitrogen use efficiency) or faster recovery after a stress pulse (e.g., woody species with higher root biomass allocation to tolerate summer drought) than native species, allowing them to become dominant in low resource environments such as arid ecosystems (Van Kleunen et al. 2010; Funk 2013). In our greenhouse experiment, buffelgrass maintained a high growth rate even under the drought watering treatment, similar to other studies (Valliere 2019; Farrell et al. 2022). In addition, buffelgrass produced a larger number of flowering culms in drier conditions, a typical drought escaping strategy (Kooyers 2015). Unexpectedly, when buffelgrass was grown with *A. purpurea* and *P. patagonica* (particularly under the drought watering treatment), buffelgrass responded by growing more, although its biomass allocation did not change. Native growth was similar across all treatments, including when grown with buffelgrass. Hence, in our controlled greenhouse conditions, buffelgrass benefitted more from the expected microclimate amelioration caused by co-occurrence (Wright et al. 2014), or by an indirect plant–soil feedback effect such as higher phosphorus mobilization (Yu et al. 2023). It is possible that the absence of observed negative drought effects on plant growth (and even higher growth in drier conditions for buffelgrass) is attributed to technical issues such as inadequate gas diffusion or anoxic effects of water saturated soils (Poorter et al. 2012a). We did not observe negative competition effects of buffelgrass on the two natives as reported in other greenhouse studies (Stevens and Fehmi 2011; Farrell et al. 2022), or dehydration symptoms and reduced photosynthetic rates in neighboring vegetation as reported in the field

Table 2 Effects of plant and watering treatments (and their interaction, P × W) on soil microbial communities

Response	Treatment	Statistic	P	R^2
Bacterial/archaeal richness	Plant	7.26	**< 0.001**	0.48
	Watering	1.44	0.234	
	P × W	1.23	0.309	
Fungal richness	Plant	2.22	0.067	0.30
	Watering	1.42	0.237	
	P × W	1.63	0.169	
Bacterial/archaeal composition	Plant	2.37	**< 0.001**	0.16
	Watering	4.50	**< 0.001**	0.06
	P × W	1.54	**< 0.001**	0.10
Fungal composition	Plant	1.96	**< 0.001**	0.14
	Watering	2.30	**< 0.001**	0.03
	P × W	1.42	**< 0.001**	0.10
Nitrogen fixers	Plant	44.44	**< 0.001**	0.86
	Watering	10.04	**0.002**	
	P × W	40.45	**< 0.001**	
Nitrifiers	Plant	23.45	**< 0.001**	0.81
	Watering	23.85	**< 0.001**	
	P × W	30.15	**< 0.001**	
Ureolytic bacteria	Plant	13.46	**0.019**	0.53
	Watering	0.80	0.370	
	P × W	18.54	**0.002**	
Methylotrophs	Plant	26.93	**< 0.001**	0.53
	Watering	0.07	0.789	
	P × W	5.51	0.357	
Chitinolytic bacteria	Plant	7.87	0.163	0.48
	Watering	4.54	**0.033**	
	P × W	15.72	**0.007**	
Cellulolytic bacteria	Plant	4.64	0.462	0.36
	Watering	0.97	0.324	
	P × W	12.65	**0.027**	
Fermenters	Plant	17.87	**0.003**	0.59
	Watering	2.66	0.103	
	P × W	18.87	**0.002**	
Fungal saprotrophs	Plant	16.03	**0.006**	0.53
	Watering	1.13	0.287	
	P × W	16.31	**0.006**	
Fungal plant pathogens	Plant	13.05	**0.023**	0.48
	Watering	0.00	0.950	
	P × W	17.41	**0.004**	

Statistic corresponds to F-statistic in the case of richness, pseudo-F in the case of composition (Bray–Curtis distance), and χ^2 in the case of functional groups. Bold values indicate significant results ($P < 0.05$). Partial R^2 (i.e., sum of squares for the variable as a proportion of the total sum of squares) are shown for PERMANOVA results

Fig. 2 Soil bacterial/archaeal (**A**) and fungal (**B**) richness across plant and watering treatments. CC refers to *Cenchrus ciliaris* (buffelgrass), AP to *Aristida purpurea* (purple three-awn), and PP to *Plantago patagonica* (woolly plantain). Shaded areas correspond to competition treatments. Bold values indicate significant results ($P < 0.05$) according to statistics in Table 2

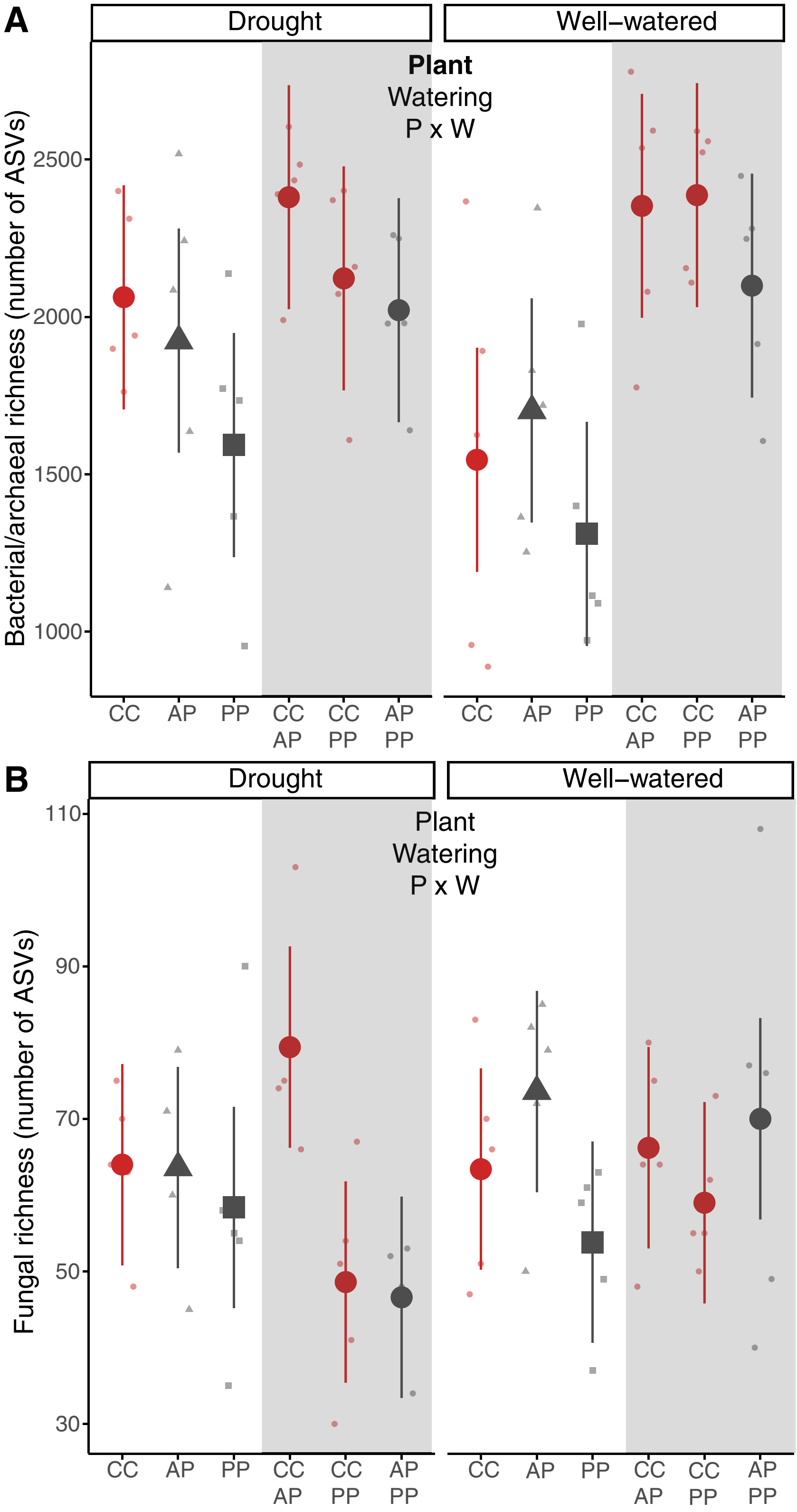
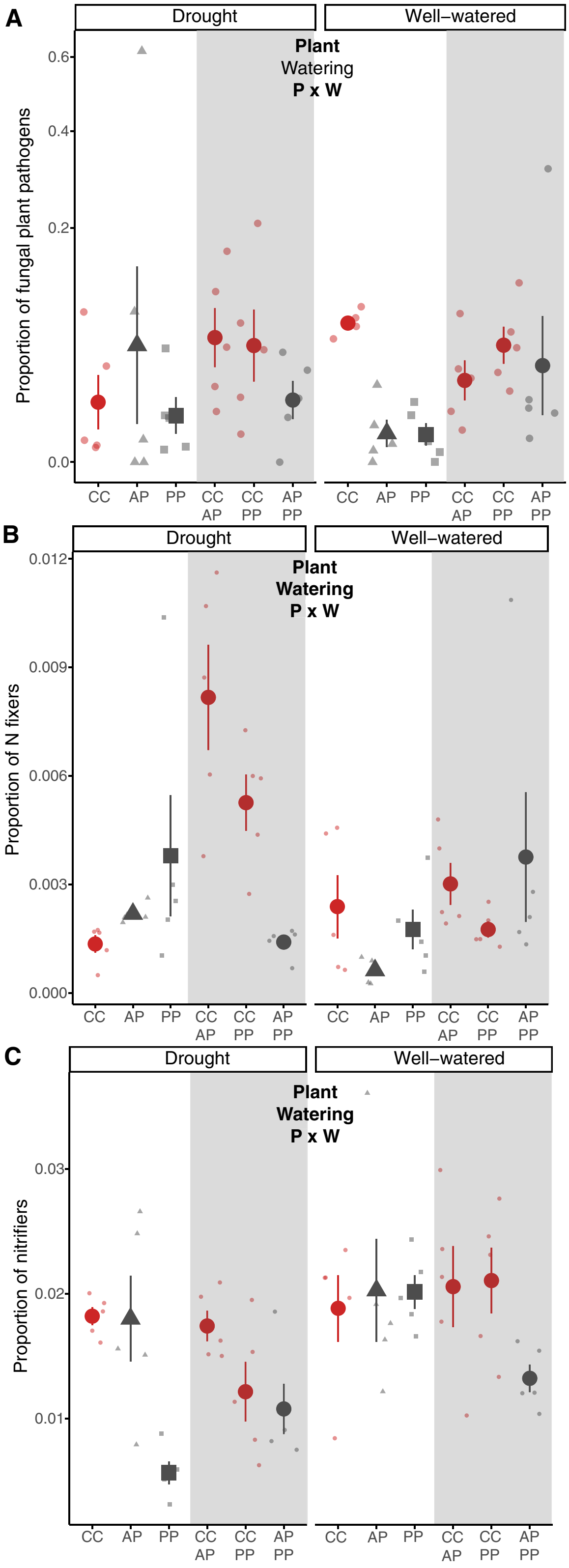


Fig. 3 Proportion of soil fungal plant pathogens (**A**), nitrogen fixers (**B**) and nitrifiers (**C**) across plant and watering treatments. CC refers to *Cenchrus ciliaris* (buffelgrass), AP to *Aristida purpurea* (purple three-awn), and PP to *Plantago patagonica* (woolly plantain). Shaded areas correspond to competition treatments. Bold values indicate significant results ($P < 0.05$) according to statistics in Table 2 ▶



(Alexander Eilts and Huxman 2013; Castellanos et al. 2016). While *A. purpurea* reduced its root allocation in competition under drier conditions and increased its root allocation in the well-watered treatment, *P. patagonica* biomass allocation was similar across treatments adding evidence to the large variability of responses to competition and drought (Lozano et al. 2020; Asefa et al. 2022). Although the ability of buffelgrass to adjust its strategy depending on its neighbor's strategy has been documented (Farrell et al. 2022), our findings show that, as is common for most invasive species, buffelgrass employs the strategy of rapid growth and accelerated lifecycle whereas native plants tend to be more conservative (Van Kleunen et al. 2010).

Effects of drought and plant competition on soil microbial communities

While water availability regulates soil microbial ecophysiology (Schimel 2018) and total biomass (Evans and Wallenstein 2012), in general, drought has little impact on soil microbial diversity (Naylor and Coleman-Derr 2018). Correspondingly, we did not observe a significant effect of watering treatments in soil bacterial or fungal diversity. On the other hand, watering treatments weakly but significantly shifted soil microbial composition. As observed in previous studies, bacterial communities were more responsive to drought than fungi (Evans and Wallenstein 2012; Barnard et al. 2013; Ochoa-Hueso et al. 2018). For example, drought shifted the rhizosphere of 18 grasses towards a higher actinobacterial abundance (Naylor et al. 2017). In our study, putative nitrifiers were more abundant in well watered treatments, while the proportion of putative nitrogen fixers was higher in drier conditions. These results are expected because low water availability inhibits nitrification in soil (Stark and Firestone 1995), and association with nitrogen fixers alleviates plant water stress (Pellegrini et al. 2016). Other symbiotic microorganisms such as arbuscular mycorrhizal fungi can also buffer the negative effects of drought (Porter et al. 2020), but as we used barren local soil and perlite (i.e., without any presence of vegetation) as soil medium, mycorrhizal fungi were essentially undetectable in our samples.

Although plant traits and resource use strategies can partially explain the success of invasive species (Van Kleunen et al. 2010), an increasing number of studies show how invasives modify soil microbial communities, with consequences for ensuing plant growth, development and survival (Reinhart and Callaway 2006; van der Putten et al. 2007; Meisner et al. 2013; Fahey et al. 2020). For example, soil biota richness generally increases following plant invasion as plant species drive the diversity of soil resources and microhabitats through complementarity in root structures and root exudate profiles (Pyšek et al. 2012). In our greenhouse experiment, buffelgrass grown with neighboring natives (i.e., *A. purpurea* and *P. patagonica*) increased soil bacterial richness but not fungal richness. Previous field studies reported increases in soil fungal richness with plant invasion (Si et al. 2013; Fahey et al. 2020). Although we hypothesized that the competition treatment would promote the abundance of fungal saprotrophs by providing a larger diversity of resources, our results show high variability across treatments and no consistent trend with plant competition.

Invasive plants may benefit through decreased pathogen exposure, increased mutualistic interactions relative to native plants or by disrupting the nitrogen cycle (Reinhart and Callaway 2006; Liao et al. 2008; Zhang et al. 2019b). In the well-watered treatment, the proportion of putative fungal plant pathogens was higher in pots where buffelgrass was present. In drier conditions, the abundance of putative fungal plant pathogens was higher when buffelgrass was grown with a native neighbor only. Thus, although these results suggest that fungal plant pathogens' abundance increase in the presence of two plant species, no consistent trend was observed in relationship to buffelgrass, reinforcing meta-analytical studies that demonstrate how plant–soil feedbacks may not be generally important as a mechanism for plant invasion (Suding et al. 2013; Meisner et al. 2014; Crawford et al. 2019).

The proportion of putative nitrogen fixers was the highest when buffelgrass was grown with a native neighbor (i.e., *A. purpurea* and *P. patagonica*) only in drier conditions. Many successful invasive plants benefit from mutualisms with nitrogen-fixing bacteria (Reinhart and Callaway 2006; Liao et al. 2008), and our results indicate that this might also be the case with buffelgrass under the conditions tested. Although invasive plants tend to be generally associated with higher nitrification rates (Hawkes et al. 2005; Liao et al. 2008), and in particular, soils from buffelgrass-invaded areas in the Sonoran Desert had a larger proportion of nitrifiers than uninvaded soil (Gornish et al. 2020), we did not observe any clear association between the presence of buffelgrass (solo or in combination) and the abundance of putative nitrifying microorganisms.

Conclusion

Understanding the interactive effects of plant invasions and drought is essential for predicting future ecosystem changes (Valliere et al. 2017). Studying plant competition and plant–soil interactions in a greenhouse setup enabled us to interpret potential mechanisms without the limitations of environmental heterogeneity in the field (Gibson et al. 1999). However, experiments conducted in the field or in the greenhouse can yield different (and even opposite) plant growth and plant–soil responses (Forero et al. 2019). For example, in this greenhouse study we observed a positive effect of buffelgrass when grown with a native on soil bacterial richness but no effects whatsoever on fungal richness, while a similar field experiment with the invasive C_4 grass *Imperata cylindrica* (cogongrass) found that soil fungal richness was affected by both invasion and drought but bacterial richness was unaffected by invasion (Fahey et al. 2020).

Buffelgrass' ability to spread in low resource habitats, outcompete native species for resources, alter fire regimes, and resist eradication is a major threat to arid ecosystems (Farrell and Gornish 2019). Our results showed that buffelgrass benefitted from growing in the vicinity of the two natives studied (i.e., *A. purpurea* and *P. patagonica*), particularly in drier conditions, and our high-throughput sequencing approach suggests that this effect might be mediated by soil bacterial richness and an increased proportion of putative nitrogen-fixing bacteria. Overall, these findings highlight the importance of considering plant–soil interactions in the fields of invasion science and restoration ecology in order to tease apart the intricacies of interactive effects of abiotic and biotic anthropogenic stressors.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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